Genome-Wide investigation of schizophrenia associated plasma Ndel1 enzyme activity

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Abbreviations: SCZ, Schizophrenia; HCs, Health Controls; GWAS, Genome-Wide Association Studies; Ndel1, Nuclear Distribution Element-Like 1; DISC1, Disrupted in Schizophrenia-1; SCID, Structured clinical interview of DSM IV; SNP, Single Nucleotide Polymorphism; BMI, Body Mass Index; CAMK1D, Calcium/calmodulin-dependent protein kinase 1D; MAGI2, Membrane-Associated Guanylate Kinase, WW and PDZ Domains-Containing 2; GABRG3, Gamma-Aminobutyric Acid Receptor, Gamma-3; CCDC25, Coiled-Coil Domain Containing Protein 25; ZNF536, Zinc Finger Protein 536; FRMPD2, FERM and PDZ domains-containing protein 2; IGF2BP3, Insulin-like growth factor 2 mRNA-binding protein 3; THSD7A, Thrombospondin, Type I, Domain Containing 7A; RNF125, Ring Finger Protein 125; OSBPL1A, Oxysterol Binding Protein-Like 1A; H3F3C, H3 Histone, Family 3C.

Joint contribution

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ABSTRACT

Ndel1 is a DISC1-interacting oligopeptidase, which cleaves neuropeptides as neurotensin and bradykinin, and has been associated with both neuronal migration and neurite outgrowth. We previously reported that plasma Ndel1 enzyme activity is lower in patients with schizophrenia (SCZ) compared to healthy controls (HCs). To our knowledge, no previous study has investigated the genetic factors associated with the plasma Ndel1 enzyme activity. In the current analyses, samples from 83 SCZ patients and 92 control subjects that were assayed for plasma Ndel1 enzyme activity were genotyped on Illumina Omni Express arrays. A genetic relationship matrix using genome-wide information was then used for ancestry correction, and association statistics were calculated genome-wide. Ndel1 enzyme activity was significantly lower in patients with SCZ ($t = 4.9$; $p < 0.001$) and was found to be associated with *CAMK1D*, *MAGI2*, *CCDC25*, and *GABGR3* at a level of suggestive significance ($p < 10^{-6}$), independent of the clinical status. Then, we performed a model to investigate the observed differences for case/control measures. 2 SNPs at region 1p22.2 reached the $p < 10^{-7}$ level. *ZFPM2* and *MAD1L1* were the only two genes with more than one hit at 10^{-6} order of p value. Therefore, Ndel1 enzyme activity is a complex trait influenced by many different genetic variants that may contribute to SCZ physiopathology.

INTRODUCTION

Schizophrenia (SCZ) is a neurodevelopmental disorder resulting from dynamic phase-specific interplays of genetic and environmental factors, which may lead to disruption of normal brain assembly and function (Murray and Lewis, 1987; Weinberger, 1987). Heritability estimates are high, *i.e.* from 73-90% (Sullivan et al., 2003) and, more recently, Genome-Wide Association Studies (GWAS) have implicated many genes and pathways in SCZ pathoetiology (Schizophrenia Working Group of the Psychiatric Genomics Consortium, 2014).

Nuclear-distribution element-like 1 (Ndel1) oligopeptidase belongs to a class of enzymes that cleaves peptides but not proteins (Barrett and Rawlings, 1992; Camargo et al., 1979). Ndel1 is a cytosolic protein able to bind and form complexes with several cytoskeleton proteins with implication in neuronal migration and neuritogenesis (Brandon et al., 2004; Hayashi et al., 2010; Kamiya et al., 2006). The *NDEL1* gene is located at chromosome 17q13.1 comprising 10 exons (Guerreiro et al., 2005), which encodes a protein with a coiled-coil domain at its N-terminal end and with oligopeptidase activity related to its C-terminal portion (Hayashi et al., 2010; Hayashi et al., 2005; Zylkiewicz et al., 2011). The full-length protein is 345 amino acids long and the enzyme active site contains a critical reactive cysteine residue at position 273 (Hayashi et al., 2010; Hayashi et al., 2005; Zylkiewicz et al., 2011). This active cysteine residue and the N-terminal coiled-coil domain were both shown to be essential for Ndel1 function (Hayashi et al., 2010; Hayashi et al., 2005; Zylkiewicz et al., 2011). Alternatively spliced transcript variants

encoding multiple isoforms were also observed for this gene (Bradshaw et al., 2009).

Several lines of evidence also support a role for Ndel1 in SCZ. Ndel1 was shown to be the major ligand of Disc1, a familial genetic liability gene for major psychiatric disorders (Brandon et al., 2004; Camargo et al., 2007). Disc1/Ndel1 interaction regulates neuronal morphogenesis and positioning during neuronal integration (Duan et al., 2007). Ndel1 and Disc1 depletion in newly generated adult hippocampal neurons share characteristics including deficits in neuronal positioning (Duan et al., 2007; Kamiya et al., 2005; Sasaki et al., 2005). Ndel1 knockdown prior to the differentiation of PC12 to neuronal cells is reported to result in inhibition of neurite outgrowth, rescued by enzymatically active wild-type Ndel1, but not by a mutant form of Ndel1 (LE266/267AA) that cannot bind Disc1 (Brandon et al., 2004) or a enzymatically-inactive mutant form of Ndel1 (C273A) that can bind Disc1 (Hayashi et al., 2010). The Disc1/Ndel1 interaction also competitively inhibits Ndel1 oligopeptidase activity (Hayashi et al., 2010). Finally, the balanced translocation disrupting the *DISC1* gene described by Millar *et al.* (2000) generates a truncated form of Disc1 protein reported to be unable to bind to Ndel1 (Hayashi et al., 2005; Kamiya et al., 2005; Pletnikov et al., 2008).

Previous studies investigating Ndel1 in SCZ have largely focused on its interaction with Disc1, with significant results only when haplotypes or interaction with Disc1 was considered (Burdick et al., 2008; Nicodemus et al., 2010). There are mixed results for Ndel1 main effects (Kahler et al., 2008; Tomppo et al., 2009). In addition, Lipska *et al.* (2006) reported a reduced

expression level of Ndel1, which was shown to be significantly influenced by *DISC1* genotype, in the hippocampus of patients.

Recently, our group showed that Ndel1 enzyme activity was significantly reduced in plasma of patients with SCZ compared to healthy controls (HCs) (Gadelha et al., 2013). Specifically, lowest Ndel1 enzyme activity values were found in more severe hebephrenic and treatment resistant patients. However, little is known about the determinants of the plasma Ndel1 enzyme activity levels. Herein, our main objective is to investigate by GWAS, the genomic variants correlated with the plasma Ndel1 enzyme activity measures.

METHODS

Study participants

In this study, 189 subjects, 87 patients with schizophrenia (SCZ) and 102 healthy controls (HCs), were enrolled. Outpatients were recruited from the Schizophrenia Program (PROESQ) of the Universidade Federal de São Paulo (UNIFESP). The Structured Clinical Interview for Diagnosis on DSM-IV (SCID) was applied by trained psychiatrists. All study participants gave informed consent for the study.

Patient inclusion criteria: 1) Between 16 and 65 years old; 2) Diagnosis of SCZ or Schizoaffective Disorder; 3) >one year of follow-up. Healthy controls were selected matching on age, gender and educational level via a governmental unemployment office. HC inclusion criteria were: 1) Age between 16 and 65 years old; 2) No current or lifetime psychiatric diagnosis; 3) Absence of known family (at any degree) history of psychosis. For both

groups exclusion criteria were: 1) Diagnosis of hypertension; 2) Use of any anti-hypertensive medication, even those prescribed for other reasons, *i.e*. propranolol for tremor; 3) Doubt or lack of consensus on diagnosis.

In the present work, 4 SCZ patients and 2 HCs were excluded due to arterial hypertension diagnosis and/or use of anti-hypertensive drugs. 8 HCs did not provide blood for DNA or the DNA quality was too low for GWAS, and therefore, they were excluded. At last, 83 patients and 92 HCs were included.

Blood samples

Blood samples were collected from all subjects into heparin vacuum tubes BD Vacutainer® (BD, NJ, USA). The samples were kept at 4°C until initial processing, and then centrifuged at 1500-2000*g* for 10-15 min to recover the plasma, which was then aliquoted and stored at -20°C in plastic microtubes (Axygen Inc., CA, USA) until use.

Activity measurements

Ndel1-like activity was measured in human plasma samples of normal HC volunteers and SCZ patients, defrosted on ice and then re-aliquoted into smaller volumes for a fluorimetric assay, using the FRET peptide substrate Abz-GFSPFRQ-EDDnp [10 µM]. Hydrolysis of the substrate, at 37°C, was monitored by measuring the fluorescence in a Shimadzu RF-5301 PC spectrofluorometer at λ_{Em} = 420 nm and λ_{Ex} = 320 nm. The 1 cm-pathlength cuvette containing 1 mL of substrate solution (50 mM Tris-HCl pH 7.4 and 100

mM NaCl buffer) was placed in a thermostatically controlled cell compartment for 5 min, before the addition of the plasma samples. The increase in fluorescence (AFU, arbitrary fluorescence units) with time was continuously recorded for 5-10 min, both in the absence and in the presence of 50 µL of a heat-inactivated Ndel1 antibody (NO_{AB} inhibitor), as previously described (Hayashi et al., 2005). Calculated Ndel1 activity was the rate of hydrolysis in the absence of the NO_{AB} inhibitor minus the rate determined in the presence of the NO_{AB} inhibitor.

Genetic Procedures

All participants were genotyped using the Illumina Human Omni Express Bead Chip (Illumina, Inc., San Diego, CA, USA). All DNA samples underwent stringent quality control including exclusion if sample genotype missing rate >1%, or if abnormal heterozygosity or unmatched sex assignment were observed. SNPs with minor allele frequency <1% or showing departure from Hardy-Weinberg equilibrium ($p < 1 \times 10^{-5}$) were excluded. The 704,523 SNPs that passed quality control were imputed to the December 2013 release from the 1000 Genomes project [1000 Genomes Consortium, 2012], using IMPUTE2 (Howie et al., 2009), with concurrent phasing (no pre-phasing). 8,793,001 SNPs with an info score > 0.8 and minor allele frequency >1% were retained for analyses.

Statistical analysis

We used GCTA Yang *et al.* (2011) to perform a genome wide analysis (GWAS) using a mixed model approach that adjusts for population stratification while carrying out association for each SNP. We derived a genetic relationship matrix (GRM) between individuals using the genetic marker genotypes on the microarrays to calculate pairwise genomic similarity. Individuals whose relationship to another subject was greater than 0.025 (equal to a fourth cousin) were removed to avoid the possible phenotypic similarity between close relatives being due to non-genetic effects, such as common environmental effects (Kang et al., 2010). Then we used GCTA's *MLMA loco* option to generate association results.

No tool based on a genetic relationship matrix was able to perform interaction analyses for genome-wide driven data. Therefore to perform this analysis we used PLINK v 1.7 (Purcell et al., 2007). To adjust for population stratification, we used a multidimensional scaling approach implemented in PLINK that gives equivalent results to principal components analysis (Price et al., 2010). The model that corrected for the first 4 dimensions provided the best estimates, considering agreement in the first 20 hits, yielded by the GCTA major effect analyses. On this basis, we carried out the interaction with case status association via a linear model, including an interaction term for case status and genotype.

Genome-wide significance threshold was defined at $p < 5 \times 10^{-8}$ (Dudbridge and Gusnanto, 2008). For non-genetic tests presented, the significance level for standard parametric and non-parametric tests are at 0.05 level.

RESULTS

No significant difference was found for age, gender or educational level between groups (Table 1). Ndel1 enzyme activity levels were significantly lower among schizophrenia (SCZ) patients, compared to healthy controls (HCs) ($t = 4.9$; $p<0.001$), as also described in our previous report (Gadelha et al., 2013). There was no significant effect of age, gender, and educational level on Ndel1 enzyme activity.

Genomewide Analysis (GWAS)

In the first set of analyses, we searched for SNPs associated with Ndel1 enzyme activity levels, independent of the case status, *e.g*. SCZ or control. Case status was then added to the linear model as a covariate. In Table 2, we report the results of analyses with p values $<10^{-6}$, and the Manhattan and QQ plots for the results are depicted (Fig. 1). Taken together, no individual SNP was above the GWAS significance threshold. We defined as suggestive significance $p < 5x10^{-6}$, as the standard practise adopted in the field. The top ranked findings was for the gene *CAMK1D*, with two hits and p values at 10^{-6} order. Other genes uncovered with $p <10^{-6}$ threshold were *MAGI2*, *GABGR3*, and *CCDC25*. The *NDEL1* gene region was found to have very few SNPs and none have reached close to significant or suggestive thresholds.

GWAS Interaction

The results for the interaction term are in Table 2, and they suggest the involvement of *DYNC1I1*, *ZFPM2*, *LINC00371*, *MAD1L1*, The most significant results were observed for the region 1p22.2 with p-value of 8.89×10^{-7}. *ZFPM2* and *MAD1L1* were the only genes with two SNPs listed above 10^{-6} threshold. The Manhattan and QQ plots for the interaction term results are depicted in Fig. 2.

DISCUSSION

We sought to understand genetic influences on Ndel1 enzyme activity, as we recently found significant lower Ndel1 enzyme activity levels in patients with schizophrenia (SCZ) compared to health controls (HCs) (Gadelha et al., 2013). In the current study, genes that bind to Ndel1 and could potentially regulate Ndel1 enzyme activity levels were investigated. We also searched for genes that significantly correlated with the observed difference in plasma Ndel1 enzyme activity levels between SCZ patients and HCs. We found no genome wide significant results, although some suggestive* hits were obtained (defining as suggestive p-values $<10^{-6}$). We found suggestive evidence for 4 genes having effects on Ndel1 enzyme activity levels: *CAMK1D*, *MAGI2*, *GABGR3*, and *CCDC25*. In our case status genotype interaction analyses, SNPs on region 1p22.2 achieved the most significant p-values and top hits were at p-level of 10^{-7}.

The *CAMK1D* gene, located at chromosome 10p13, encompassing the loci with the lowest p-values had a number of suggestive association signals, not in linkage disequilibrium, at p-values $<10^{-6}$. CAMk1D is a Ca^{2+}/calmodulin dependent kinase predominantly expressed in polymorphonuclear cells, among hematopoietic cell lines (Verploegen et al., 2000). In *in vitro* assays, CAMk1D phosphorylated several substrates, including synapsin 1 and 2, and CREB (Nairn and Greengard, 1987; Sheng et al., 1991), which were previously associated with SCZ (Fei et al., 2010; Kawanishi et al., 1999; Saviouk et al., 2007). The binding of Ca^{2+} to calmodulin is a signal transducing mechanism of Ca^{2+} into cellular responses that regulate many

crucial processes (Chin and Means, 2000). Ripke *et al.* (2013) emphasized the potential role of Ca^{2+} neuronal signalling to SCZ via the observed associations at several calcium channel subunit genes, including *CACNA1C* and *CACNA1D*.

CAMk1D has been reported to be associated in GWAS meta-analysis studies with type II Diabetes (Zeggini et al., 2008) and was later shown to reduce glucose response (Grarup et al., 2008), possibly due to an effect on the localization of a mediator of both gluconeogenesis and glycolysis (Haney et al., 2013). This raises the question about the effect of metabolic process in Ndel1 measures and whether they converge to impact on SCZ pathophysiology or whether Ndel1 would be only a biomarker of these processes. In our first study (Gadelha et al., 2013), we did not observe any association between Ndel1 enzyme activity and Body Mass Index (BMI) in a subgroup of patients, nor with antipsychotic drug doses in chlorpromazine equivalents. However, one cannot rule out any of these possibilities, and they should be addressed in future studies.

MAGI2 has been implicated in both glutamatergic (Deng et al., 2006) and GABAergic pathways (Sassoe-Pognetto et al., 2011). SNPs of *MAGI2* have been associated with performance in the Wisconsin Sorting Card Test (WCST) in patients with SCZ (Koide et al., 2012), whereas in animal models it was shown to modulate long-term memory and associative learning (Emtage et al., 2009; Stetak et al., 2009).

The *GABGR3* gene encodes a subunit of the GABA-A receptor and is located at chromosome 15q12, inside a locus containing a cluster of other two

GABA(A) receptor subunit genes, *GABRB3* and *GABRA5*. Despite substantive evidence of impaired GABA neurotransmission in SCZ (Wassef et al., 2003), no previous study has directly associated *GABGR3* to the disorder. The 15q11-13 locus has been identified in several CNV studies in SCZ as well as in a familial SCZ linkage study (Giusti-Rodriguez and Sullivan, 2013; Liao et al., 2012). Kohannim *et al.* (2012) reported 22 genes associated with temporal lobe volume and among them were *GABRG3* and *MAGI2*.

Our analyses of case control differences using an interaction term underwent less stringent ancestry correction and should be interpreted with caution. The most significant results were in the 1p22.2 region. A CNV on this region has been associated to neurodevelopmental phenotype (Girirajan et al., 2011). 1p22 is in a chromosome fragile site, which is believed to constitute areas of chromatin that fail to compact during mitosis (Lukusa and Fryns, 2008). Some recent studies suggested the association between *MAD1L1* and SCZ and bipolar disorder (Jia et al., 2012; Ruderfer et al., 2014), including one of the largest GWAS on SCZ (Ripke et al., 2013). A *ZFPM2* polymorphism (rs12678719) showed to modulate the risk to develop antipsychotic-induced parkinsonism in patients with SCZ (Greenbaum et al., 2012). A point mutation on *DYNC1I1* resulted in altered neuronal development and elevated anxiety levels in animal model (Banks et al., 2011). We did not find any link between *LINC00371* and SCZ.

Considering both set of analyses, several of the genes uncovered by us here were previously reported to be expressed or associated with immune cells (*e.g.CAMK1D*, *MAGI2*), which possibly also suggests the origin of the Ndel1 found in plasma (Paik et al., 1992). In the interaction analyses,

MAD1L1 seems the most promising result for being previously associated in a GWAS of SCZ.

Our small sample size to perform GWAS analyses is the major limitation of this work. However, enzyme levels may be a more robust phenotype than psychiatric categorical phenotypes. For our analysis of Ndel1 level, the lack of significant SNPs tagging the Ndel1 locus indicates that Ndel1 level is likely to be a complex, perhaps polygenic, trait. Ndel1 is also essential for normal brain development and severe disruption of the protein structure or reduction on its physiological levels leads to intrauterine abortion (Sasaki et al., 2005). This could explain why Ndel1 enzyme levels appear to a more complex trait than, for example, angiotensin I converting enzyme (ACE), other oligopeptidase that share common peptide substrates with Ndel1 (Terao et al., 2013).

In a previous study, we worked on ruling out confounding factors, and showed that Ndel1 levels were not correlated to the dose of antipsychotics, BMI or cigarette smoking (Gadelha et al., 2013). Multiple ancestry admixture is a well-know problem dealing with Brazilian samples. For this reason, we used GCTA to generate kinship matrix using information in the array to perform ancestry correction. It was not possible to apply the same approach to the interaction analyses, but of note, our finding of best correction to 4 principal components is in agreement with a report from Kang *et al.* (2010), where inclusion of 2 to 5 principal components had relevant impact on the inflation factor and further augmenting did not result in better genomic control of statistical measures. Finally, even with correction for clinical status and

medication on the main effects analysis, we also cannot rule out that some of the findings were driven by SCZ diagnosis.

Lastly, the genome-wide approach employed here allowed us an unbiased investigation on genetic influence on plasma Ndel1 enzyme activity measures with new putative genes possibly implicated in SCZ. The relatively high significance of genetic variants coding Ndel1 enzyme activity suggests that a more precise physiopathology driven hypothesis may increase the chances of finding relevant genes to SCZ. Future larger studies focused on understanding the involvement of such genes may help to clarify the role of Ndel1 expression and activity in SCZ pathophysiology.

REFERENCES

Banks, G.T., Haas, M.A., Line, S., Shepherd, H.L., Alqatari, M., Stewart, S., Rishal, I., Philpott, A., Kalmar, B., Kuta, A., Groves, M., Parkinson, N., Acevedo-Arozena, A., Brandner, S., Bannerman, D., Greensmith, L., Hafezparast, M., Koltzenburg, M., Deacon, R., Fainzilber, M., Fisher, E.M., 2011. Behavioral and other phenotypes in a cytoplasmic Dynein light intermediate chain 1 mutant mouse. J Neurosci 31(14), 5483-5494.

Barrett, A.J., Rawlings, N.D., 1992. Oligopeptidases, and the emergence of the prolyl oligopeptidase family. Biol Chem Hoppe Seyler 373(7), 353-360.

Bradshaw, N.J., Christie, S., Soares, D.C., Carlyle, B.C., Porteous, D.J., Millar, J.K., 2009. NDE1 and NDEL1: multimerisation, alternate splicing and DISC1 interaction. Neurosci Lett 449(3), 228-233.

Brandon, N.J., Handford, E.J., Schurov, I., Rain, J.C., Pelling, M., Duran-Jimeniz, B., Camargo, L.M., Oliver, K.R., Beher, D., Shearman, M.S., Whiting, P.J., 2004. Disrupted in Schizophrenia 1 and Nudel form a neurodevelopmentally regulated protein complex: implications for schizophrenia and other major neurological disorders. Mol Cell Neurosci 25(1), 42-55.

Burdick, K.E., Kamiya, A., Hodgkinson, C.A., Lencz, T., DeRosse, P., Ishizuka, K., Elashvili, S., Arai, H., Goldman, D., Sawa, A., Malhotra, A.K., 2008. Elucidating the relationship between DISC1, NDEL1 and NDE1 and the risk for schizophrenia: evidence of epistasis and competitive binding. Hum Mol Genet 17(16), 2462-2473.

Camargo, A.C., Caldo, H., Reis, M.L., 1979. Susceptibility of a peptide derived from bradykinin to hydrolysis by brain endo-oligopeptidases and pancreatic proteinases. J Biol Chem 254(12), 5304-5307.

Camargo, L.M., Collura, V., Rain, J.C., Mizuguchi, K., Hermjakob, H., Kerrien, S., Bonnert, T.P., Whiting, P.J., Brandon, N.J., 2007. Disrupted in Schizophrenia 1 Interactome: evidence for the close connectivity of risk genes and a potential synaptic basis for schizophrenia. Mol Psychiatry 12(1), 74-86.

Chin, D., Means, A.R., 2000. Calmodulin: a prototypical calcium sensor. Trends Cell Biol 10(8), 322-328.

Deng, F., Price, M.G., Davis, C.F., Mori, M., Burgess, D.L., 2006. Stargazin and other transmembrane AMPA receptor regulating proteins interact with synaptic scaffolding protein MAGI-2 in brain. J Neurosci 26(30), 7875-7884.

Duan, X., Chang, J.H., Ge, S., Faulkner, R.L., Kim, J.Y., Kitabatake, Y., Liu, X.B., Yang, C.H., Jordan, J.D., Ma, D.K., Liu, C.Y., Ganesan, S., Cheng, H.J., Ming, G.L., Lu, B., Song, H., 2007. Disrupted-In-Schizophrenia 1 regulates integration of newly generated neurons in the adult brain. Cell 130(6), 1146-1158.

Dudbridge, F., Gusnanto, A., 2008. Estimation of significance thresholds for genomewide association scans. Genet Epidemiol 32(3), 227-234.

Emtage, L., Chang, H., Tiver, R., Rongo, C., 2009. MAGI-1 modulates AMPA receptor synaptic localization and behavioral plasticity in response to prior experience. PLoS One 4(2), e4613.

Fei, E., Ma, X., Zhu, C., Xue, T., Yan, J., Xu, Y., Zhou, J., Wang, G., 2010. Nucleocytoplasmic shuttling of dysbindin-1, a schizophrenia-related protein, regulates synapsin I expression. J Biol Chem 285(49), 38630-38640.

Gadelha, A., Machado, M.F., Yonamine, C.M., Sato, J.R., Juliano, M.A., Oliveira, V., Bressan, R.A., Hayashi, M.A., 2013. Plasma Ndel1 enzyme activity is reduced in patients with schizophrenia--a potential biomarker? J Psychiatr Res 47(5), 657-663.

Girirajan, S., Brkanac, Z., Coe, B.P., Baker, C., Vives, L., Vu, T.H., Shafer, N., Bernier, R., Ferrero, G.B., Silengo, M., Warren, S.T., Moreno, C.S., Fichera, M., Romano, C., Raskind, W.H., Eichler, E.E., 2011. Relative burden of large CNVs on a range of neurodevelopmental phenotypes. PLoS Genet 7(11), e1002334.

Giusti-Rodriguez, P., Sullivan, P.F., 2013. The genomics of schizophrenia: update and implications. J Clin Invest 123(11), 4557-4563.

Grarup, N., Andersen, G., Krarup, N.T., Albrechtsen, A., Schmitz, O., Jorgensen, T., Borch-Johnsen, K., Hansen, T., Pedersen, O., 2008. Association testing of novel type 2 diabetes risk alleles in the JAZF1, CDC123/CAMK1D, TSPAN8, THADA, ADAMTS9, and NOTCH2 loci with insulin release, insulin sensitivity, and obesity in a population-based sample of 4,516 glucose-tolerant middle-aged Danes. Diabetes 57(9), 2534-2540.

Greenbaum, L., Smith, R.C., Lorberboym, M., Alkelai, A., Zozulinsky, P., Lifschytz, T., Kohn, Y., Djaldetti, R., Lerer, B., 2012. Association of the ZFPM2 gene with antipsychotic-induced parkinsonism in schizophrenia patients. Psychopharmacology (Berl) 220(3), 519-528.

Guerreiro, J.R., Winnischofer, S.M., Bastos, M.F., Portaro, F.C., Sogayar, M.C., de Camargo, A.C., Hayashi, M.A., 2005. Cloning and characterization of the human and rabbit NUDEL-oligopeptidase promoters and their negative regulation. Biochim Biophys Acta 1730(1), 77-84.

Haney, S., Zhao, J., Tiwari, S., Eng, K., Guey, L.T., Tien, E., 2013. RNAi screening in primary human hepatocytes of genes implicated in genome-wide association studies for roles in type 2 diabetes identifies roles for CAMK1D and CDKAL1, among others, in hepatic glucose regulation. PLoS One 8(6), e64946.

Hayashi, M.A., Guerreiro, J.R., Charych, E., Kamiya, A., Barbosa, R.L., Machado, M.F., Campeiro, J.D., Oliveira, V., Sawa, A., Camargo, A.C., Brandon, N.J., 2010. Assessing the role of endooligopeptidase activity of Ndel1 (nuclear-distribution gene E homolog like-1) in neurite outgrowth. Mol Cell Neurosci 44(4), 353-361.

Hayashi, M.A., Portaro, F.C., Bastos, M.F., Guerreiro, J.R., Oliveira, V., Gorrao, S.S., Tambourgi, D.V., Sant'Anna, O.A., Whiting, P.J., Camargo, L.M., Konno, K., Brandon, N.J., Camargo, A.C., 2005. Inhibition of NUDEL (nuclear distribution element-like)-oligopeptidase activity by disrupted-in-schizophrenia 1. Proc Natl Acad Sci U S A 102(10), 3828-3833.

Howie, B.N., Donnelly, P., Marchini, J., 2009. A flexible and accurate genotype imputation method for the next generation of genome-wide association studies. PLoS Genet 5(6), e1000529.

Jia, P., Wang, L., Fanous, A.H., Pato, C.N., Edwards, T.L., Zhao, Z., 2012. Network-assisted investigation of combined causal signals from genome-wide association studies in schizophrenia. PLoS Comput Biol 8(7), e1002587.

Kahler, A.K., Djurovic, S., Kulle, B., Jonsson, E.G., Agartz, I., Hall, H., Opjordsmoen, S., Jakobsen, K.D., Hansen, T., Melle, I., Werge, T., Steen, V.M., Andreassen, O.A., 2008. Association analysis of schizophrenia on 18 genes involved in neuronal migration: MDGA1 as a new susceptibility gene. Am J Med Genet B Neuropsychiatr Genet 147B(7), 1089-1100.

Kamiya, A., Kubo, K., Tomoda, T., Takaki, M., Youn, R., Ozeki, Y., Sawamura, N., Park, U., Kudo, C., Okawa, M., Ross, C.A., Hatten, M.E.,

Nakajima, K., Sawa, A., 2005. A schizophrenia-associated mutation of DISC1 perturbs cerebral cortex development. Nat Cell Biol 7(12), 1167-1178.

Kamiya, A., Tomoda, T., Chang, J., Takaki, M., Zhan, C., Morita, M., Cascio, M.B., Elashvili, S., Koizumi, H., Takanezawa, Y., Dickerson, F., Yolken, R., Arai, H., Sawa, A., 2006. DISC1-NDEL1/NUDEL protein interaction, an essential component for neurite outgrowth, is modulated by genetic variations of DISC1. Hum Mol Genet 15(22), 3313-3323.

Kang, H.M., Sul, J.H., Service, S.K., Zaitlen, N.A., Kong, S.Y., Freimer, N.B., Sabatti, C., Eskin, E., 2010. Variance component model to account for sample structure in genome-wide association studies. Nat Genet 42(4), 348-354.

Kawanishi, Y., Harada, S., Tachikawa, H., Okubo, T., Shiraishi, H., 1999. Novel variants in the promoter region of the CREB gene in schizophrenic patients. J Hum Genet 44(6), 428-430.

Kohannim, O., Hibar, D.P., Stein, J.L., Jahanshad, N., Hua, X., Rajagopalan, P., Toga, A.W., Jack, C.R., Jr., Weiner, M.W., de Zubicaray, G.I., McMahon, K.L., Hansell, N.K., Martin, N.G., Wright, M.J., Thompson, P.M., 2012. Discovery and Replication of Gene Influences on Brain Structure Using LASSO Regression. Front Neurosci 6, 115.

Koide, T., Banno, M., Aleksic, B., Yamashita, S., Kikuchi, T., Kohmura, K., Adachi, Y., Kawano, N., Kushima, I., Nakamura, Y., Okada, T., Ikeda, M., Ohi, K., Yasuda, Y., Hashimoto, R., Inada, T., Ujike, H., Iidaka, T., Suzuki, M., Takeda, M., Iwata, N., Ozaki, N., 2012. Common variants in MAGI2 gene are associated with increased risk for cognitive impairment in schizophrenic patients. PLoS One 7(5), e36836.

Liao, H.M., Chao, Y.L., Huang, A.L., Cheng, M.C., Chen, Y.J., Lee, K.F., Fang, J.S., Hsu, C.H., Chen, C.H., 2012. Identification and characterization of three inherited genomic copy number variations associated with familial schizophrenia. Schizophr Res 139(1-3), 229-236.

Lipska, B.K., Peters, T., Hyde, T.M., Halim, N., Horowitz, C., Mitkus, S., Weickert, C.S., Matsumoto, M., Sawa, A., Straub, R.E., Vakkalanka, R., Herman, M.M., Weinberger, D.R., Kleinman, J.E., 2006. Expression of DISC1 binding partners is reduced in schizophrenia and associated with DISC1 SNPs. Hum Mol Genet 15(8), 1245-1258.

Lukusa, T., Fryns, J.P., 2008. Human chromosome fragility. Biochim Biophys Acta 1779(1), 3-16.

Millar, J.K., Wilson-Annan, J.C., Anderson, S., Christie, S., Taylor, M.S., Semple, C.A., Devon, R.S., St Clair, D.M., Muir, W.J., Blackwood, D.H., Porteous, D.J., 2000. Disruption of two novel genes by a translocation co-segregating with schizophrenia. Hum Mol Genet 9(9), 1415-1423.

Murray, R.M., Lewis, S.W., 1987. Is schizophrenia a neurodevelopmental disorder? Br Med J (Clin Res Ed) 295(6600), 681-682.

Nairn, A.C., Greengard, P., 1987. Purification and characterization of Ca2+/calmodulin-dependent protein kinase I from bovine brain. J Biol Chem 262(15), 7273-7281.

Nicodemus, K.K., Callicott, J.H., Higier, R.G., Luna, A., Nixon, D.C., Lipska, B.K., Vakkalanka, R., Giegling, I., Rujescu, D., St Clair, D., Muglia, P., Shugart, Y.Y., Weinberger, D.R., 2010. Evidence of statistical epistasis between DISC1, CIT and NDEL1 impacting risk for schizophrenia: biological validation with functional neuroimaging. Hum Genet 127(4), 441-452.

Paik, S.H., Betti, F., Camargo, A.C., de Oliveira, E.S., 1992. Presence of endo-oligopeptidase (EC 3.4.22.19), a putative neuropeptide-metabolizing endopeptidase in cells of the immune system. J Neuroimmunol 38(1-2), 35-44.

Pletnikov, M.V., Ayhan, Y., Nikolskaia, O., Xu, Y., Ovanesov, M.V., Huang, H., Mori, S., Moran, T.H., Ross, C.A., 2008. Inducible expression of mutant human DISC1 in mice is associated with brain and behavioral abnormalities reminiscent of schizophrenia. Mol Psychiatry 13(2), 173-186, 115.

Price, A.L., Zaitlen, N.A., Reich, D., Patterson, N., 2010. New approaches to population stratification in genome-wide association studies. Nat Rev Genet 11(7), 459-463.

Purcell, S., Neale, B., Todd-Brown, K., Thomas, L., Ferreira, M.A., Bender, D., Maller, J., Sklar, P., de Bakker, P.I., Daly, M.J., Sham, P.C., 2007. PLINK: a tool set for whole-genome association and population-based linkage analyses. Am J Hum Genet 81(3), 559-575.

Ripke, S., O'Dushlaine, C., Chambert, K., Moran, J.L., Kahler, A.K., Akterin, S., Bergen, S.E., Collins, A.L., Crowley, J.J., Fromer, M., Kim, Y., Lee, S.H., Magnusson, P.K., Sanchez, N., Stahl, E.A., Williams, S., Wray, N.R., Xia, K., Bettella, F., Borglum, A.D., Bulik-Sullivan, B.K., Cormican, P., Craddock, N., de Leeuw, C., Durmishi, N., Gill, M., Golimbet, V., Hamshere, M.L., Holmans, P., Hougaard, D.M., Kendler, K.S., Lin, K., Morris, D.W., Mors, O., Mortensen, P.B., Neale, B.M., O'Neill, F.A., Owen, M.J., Milovancevic, M.P., Posthuma, D., Powell, J., Richards, A.L., Riley, B.P., Ruderfer, D., Rujescu, D., Sigurdsson, E., Silagadze, T., Smit, A.B., Stefansson, H., Steinberg, S., Suvisaari, J., Tosato, S., Verhage, M., Walters, J.T., Levinson, D.F., Gejman, P.V., Laurent, C., Mowry, B.J., O'Donovan, M.C., Pulver, A.E., Schwab, S.G., Wildenauer, D.B., Dudbridge, F., Shi, J., Albus, M., Alexander, M., Campion, D., Cohen, D., Dikeos, D., Duan, J., Eichhammer, P., Godard, S., Hansen, M., Lerer, F.B., Liang, K.Y., Maier, W., Mallet, J., Nertney, D.A., Nestadt, G., Norton, N., Papadimitriou, G.N., Ribble, R., Sanders, A.R., Silverman, J.M., Walsh, D., Williams, N.M., Wormley, B., Arranz, M.J., Bakker, S., Bender, S., Bramon, E., Collier, D., Crespo-Facorro, B., Hall, J., Iyegbe, C., Jablensky, A., Kahn, R.S., Kalaydjieva, L., Lawrie, S., Lewis, C.M., Linszen, D.H., Mata, I., McIntosh, A., Murray, R.M., Ophoff, R.A., Van Os, J., Walshe, M., Weisbrod, M., Wiersma, D., Donnelly, P., Barroso, I., Blackwell, J.M., Brown, M.A., Casas, J.P., Corvin, A.P., Deloukas, P., Duncanson, A., Jankowski, J., Markus, H.S., Mathew, C.G., Palmer, C.N., Plomin, R., Rautanen, A., Sawcer,

S.J., Trembath, R.C., Viswanathan, A.C., Wood, N.W., Spencer, C.C., Band, G., Bellenguez, C., Freeman, C., Hellenthal, G., Giannoulatou, E., Pirinen, M., Pearson, R.D., Strange, A., Su, Z., Vukcevic, D., Langford, C., Hunt, S.E., Edkins, S., Gwilliam, R., Blackburn, H., Bumpstead, S.J., Dronov, S., Gillman, M., Gray, E., Hammond, N., Jayakumar, A., McCann, O.T., Liddle, J., Potter, S.C., Ravindrarajah, R., Ricketts, M., Tashakkori-Ghanbaria, A., Waller, M.J., Weston, P., Widaa, S., Whittaker, P., McCarthy, M.I., Stefansson, K., Scolnick, E., Purcell, S., McCarroll, S.A., Sklar, P., Hultman, C.M., Sullivan, P.F., 2013. Genome-wide association analysis identifies 13 new risk loci for schizophrenia. Nat Genet 45(10), 1150-1159.

Ruderfer, D.M., Fanous, A.H., Ripke, S., McQuillin, A., Amdur, R.L., Gejman, P.V., O'Donovan, M.C., Andreassen, O.A., Djurovic, S., Hultman, C.M., Kelsoe, J.R., Jamain, S., Landen, M., Leboyer, M., Nimgaonkar, V., Nurnberger, J., Smoller, J.W., Craddock, N., Corvin, A., Sullivan, P.F., Holmans, P., Sklar, P., Kendler, K.S., 2014. Polygenic dissection of diagnosis and clinical dimensions of bipolar disorder and schizophrenia. Mol Psychiatry 19(9), 1017-1024.

Sasaki, S., Mori, D., Toyo-oka, K., Chen, A., Garrett-Beal, L., Muramatsu, M., Miyagawa, S., Hiraiwa, N., Yoshiki, A., Wynshaw-Boris, A., Hirotsune, S., 2005. Complete loss of Ndel1 results in neuronal migration defects and early embryonic lethality. Mol Cell Biol 25(17), 7812-7827.

Sassoe-Pognetto, M., Frola, E., Pregno, G., Briatore, F., Patrizi, A., 2011. Understanding the molecular diversity of GABAergic synapses. Front Cell Neurosci 5, 4.

Saviouk, V., Moreau, M.P., Tereshchenko, I.V., Brzustowicz, L.M., 2007. Association of synapsin 2 with schizophrenia in families of Northern European ancestry. Schizophr Res 96(1-3), 100-111.

Schizophrenia Working Group of the Psychiatric Genomics Consortium, 2014. Biological insights from 108 schizophrenia-associated genetic loci. Nature 511(7510), 421-427.

Sheng, M., Thompson, M.A., Greenberg, M.E., 1991. CREB: a Ca(2+)-regulated transcription factor phosphorylated by calmodulin-dependent kinases. Science 252(5011), 1427-1430.

Stetak, A., Horndli, F., Maricq, A.V., van den Heuvel, S., Hajnal, A., 2009. Neuron-specific regulation of associative learning and memory by MAGI-1 in C. elegans. PLoS One 4(6), e6019.

Sullivan, P.F., Kendler, K.S., Neale, M.C., 2003. Schizophrenia as a complex trait: evidence from a meta-analysis of twin studies. Arch Gen Psychiatry 60(12), 1187-1192.

Terao, C., Bayoumi, N., McKenzie, C.A., Zelenika, D., Muro, S., Mishima, M., Connell, J.M., Vickers, M.A., Lathrop, G.M., Farrall, M., Matsuda, F., Keavney, B.D., 2013. Quantitative variation in plasma angiotensin-I converting enzyme

activity shows allelic heterogeneity in the ABO blood group locus. Ann Hum Genet 77(6), 465-471.

Tomppo, L., Hennah, W., Lahermo, P., Loukola, A., Tuulio-Henriksson, A., Suvisaari, J., Partonen, T., Ekelund, J., Lonnqvist, J., Peltonen, L., 2009. Association between genes of Disrupted in schizophrenia 1 (DISC1) interactors and schizophrenia supports the role of the DISC1 pathway in the etiology of major mental illnesses. Biol Psychiatry 65(12), 1055-1062.

Verploegen, S., Lammers, J.W., Koenderman, L., Coffer, P.J., 2000. Identification and characterization of CKLiK, a novel granulocyte Ca(++)/calmodulin-dependent kinase. Blood 96(9), 3215-3223.

Wassef, A., Baker, J., Kochan, L.D., 2003. GABA and schizophrenia: a review of basic science and clinical studies. J Clin Psychopharmacol 23(6), 601-640.

Weinberger, D.R., 1987. Implications of normal brain development for the pathogenesis of schizophrenia. Arch Gen Psychiatry 44(7), 660-669.

Yang, J., Lee, S.H., Goddard, M.E., Visscher, P.M., 2011. GCTA: a tool for genome-wide complex trait analysis. Am J Hum Genet 88(1), 76-82.

Zeggini, E., Scott, L.J., Saxena, R., Voight, B.F., Marchini, J.L., Hu, T., de Bakker, P.I., Abecasis, G.R., Almgren, P., Andersen, G., Ardlie, K., Bostrom, K.B., Bergman, R.N., Bonnycastle, L.L., Borch-Johnsen, K., Burtt, N.P., Chen, H., Chines, P.S., Daly, M.J., Deodhar, P., Ding, C.J., Doney, A.S., Duren, W.L., Elliott, K.S., Erdos, M.R., Frayling, T.M., Freathy, R.M., Gianniny, L., Grallert, H., Grarup, N., Groves, C.J., Guiducci, C., Hansen, T., Herder, C., Hitman, G.A., Hughes, T.E., Isomaa, B., Jackson, A.U., Jorgensen, T., Kong, A., Kubalanza, K., Kuruvilla, F.G., Kuusisto, J., Langenberg, C., Lango, H., Lauritzen, T., Li, Y., Lindgren, C.M., Lyssenko, V., Marvelle, A.F., Meisinger, C., Midthjell, K., Mohlke, K.L., Morken, M.A., Morris, A.D., Narisu, N., Nilsson, P., Owen, K.R., Palmer, C.N., Payne, F., Perry, J.R., Pettersen, E., Platou, C., Prokopenko, I., Qi, L., Qin, L., Rayner, N.W., Rees, M., Roix, J.J., Sandbaek, A., Shields, B., Sjogren, M., Steinthorsdottir, V., Stringham, H.M., Swift, A.J., Thorleifsson, G., Thorsteinsdottir, U., Timpson, N.J., Tuomi, T., Tuomilehto, J., Walker, M., Watanabe, R.M., Weedon, M.N., Willer, C.J., Illig, T., Hveem, K., Hu, F.B., Laakso, M., Stefansson, K., Pedersen, O., Wareham, N.J., Barroso, I., Hattersley, A.T., Collins, F.S., Groop, L., McCarthy, M.I., Boehnke, M., Altshuler, D., 2008. Meta-analysis of genome-wide association data and large-scale replication identifies additional susceptibility loci for type 2 diabetes. Nat Genet 40(5), 638-645.

Zylkiewicz, E., Kijanska, M., Choi, W.C., Derewenda, U., Derewenda, Z.S., Stukenberg, P.T., 2011. The N-terminal coiled-coil of Ndel1 is a regulated scaffold that recruits LIS1 to dynein. J Cell Biol 192(3), 433-445.

LEGENDS

Figure 1. Results of Ndel1 enzyme activity versus GWAS – major effects. A) Manhattan Plot depicting observed p-values by chromosome. B) QQ plot of observed x expected p-values.

Figure 2. Results of Ndel1 enzyme activity versus GWAS – interaction term results. A) Manhattan Plot depicting observed p values by chromosome. B) QQ plot of observed versus expected p-values.

TABLES

Table 1. Sociodemographic characteristics.

		Patients (n=83)		Healthy Controls (n=92)		Statistics	
		N	(%)	N	(%)	Test value	p-value
Gender	Male	56	(67.5)	60	(65.2)	0.011	0.753
	Female	27	(32.5)	32	(34.8)		
Educational level	>10[a]	24	(28.9)	27	(29.3)	0.004	0.950
	<10[a]	59	(71.1)	65	(70.7)		
Age, Mean	Years	86	(34.92)	100	-34	-0.612	0.541

a = years of education

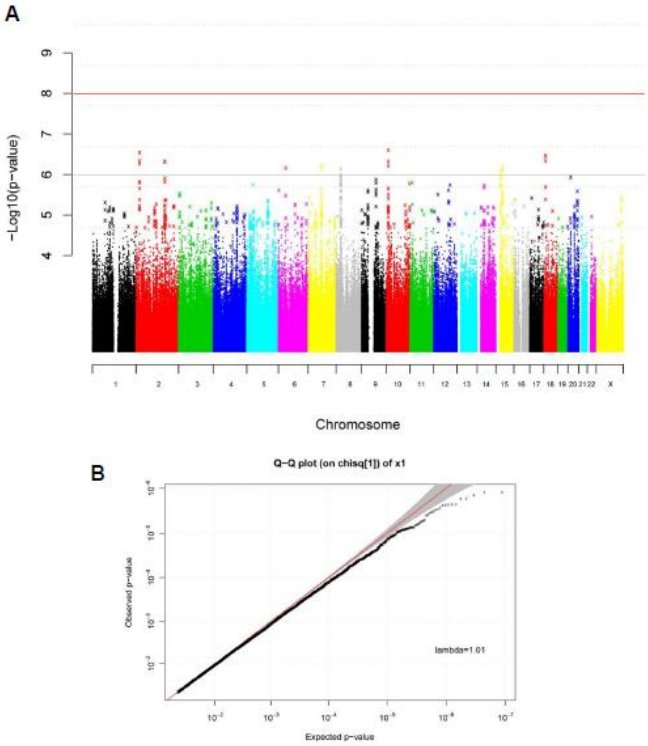
Table 2. Top Results for genome-wide investigation of Ndel1 plasma

SNP	Region	Beta	P-value	Gene
rs10184029	2p24	-0.535205	2.78×10^{-6}	No gene
rs4495787	10p13	-0.322829	2.88×10^{-6}	CAMK1D
rs63439260	10p13	-0.330936	2.91×10^{-6}	CAMK1D
rs1190669	18p11	-0.335355	3.37×10^{-6}	No gene
rs564499	18p11	-0.329733	3.46×10^{-6}	No gene
rs515503	18p11	-0.334274	4.39×10^{-6}	No gene
rs62111833	2p24	-0.642179	4.84×10^{-6}	No gene
rs72787916	2p24	-0.59675	5.39×10^{-6}	No gene
rs7902494	10p13	-0.307938	5.53×10^{-6}	CAMK1D
rs2704367	2q24.3	0.29847	5.69×10^{-6}	No gene
rs2615325	2q24.3	0.298237	5.79×10^{-6}	No gene
rs8041484	15q24	-0.36779	5.87×10^{-6}	No gene
rs17439060	7q21	0.3279	6.12×10^{-6}	MAGI2
rs2941397	6p21	-0.37646	6.30×10^{-6}	No gene
rs17439102	7q21	0.299595	6.69×10^{-6}	MAGI2
rs10906183	10p13	-0.31853	6.76×10^{-6}	CAMK1D
rs1075587	8p21	-0.344483	7.22×10^{-6}	No gene
rs59405048	15q12	-0.363503	7.23×10^{-6}	GABRG3
rs140459790	15q12	-0.354705	7.86×10^{-6}	GABRG3
rs35767880	8p21.1	-0.324939	9.17×10^{-6}	CCDC25
rs4887557	15q12	-0.365408	9.51×10^{-6}	GABRG3

Table 3. Top results for genome-wide results for Ndel1 plasma enzyme activity/clinical status interaction.

CHR	SNP	BP	A1	Beta	Stat	P	Region	Gene
1	rs4526597	91064319	C	-0.7466	-5.173	6,91E-07	1p22.2	No gene
1	rs479169	91035731	C	-0.7682	-5.129	8,89E-07	1p22.2	No gene
1	rs7418693	91068580	T	-0.7234	-5.017	1,39E-06	1p22.2	No gene
1	rs6428591	91066089	A	-0.7177	-4.972	1,70E-06	1p22.2	No gene
1	rs10801818	91068211	T	-0.7092	-4.924	2,12E-06	1p22.2	No gene
11	rs1752232	35875195	T	-1.056	-4.989	2,18E-06	11p13	No gene
10	rs4526672	66926880	T	0.5951	4.851	2,90E-06	10q21.3	No gene
1	rs10922882	91053707	C	-0.6918	-4.82	3,27E-06	1p22.2	No gene
7	rs7808673	95532968	T	-0.6968	-4.782	4,01E-06	7q21.3	DYNC1I1
8	rs7836133	106747160	T	-0.607	-4.76	4,26E-06	8q23.1	ZFPM2
7	rs4721188	1953615	G	-0.7879	-4.747	4,75E-06	7p22.3	MAD1L1
13	rs142345263	51746239	G	0.7771	4.758	4,93E-06	13q14.3	LINC00371
10	rs4745861	66927624	G	-0.627	-4.725	5,10E-06	10q21.3	No gene
8	rs11997907	19992955	A	0.741	4.712	5,24E-06	8p21.3	No gene
8	rs10808406	106724356	G	-0.6173	-4.709	5,57E-06	8q23.1	ZFPM2
10	rs4317873	66925741	T	-0.6239	-4.686	6,00E-06	10q21.3	No gene
7	rs111698506	1977834	A	-0.8773	-4.682	6.67e-06	7p22.3	MAD1L1
12	rs78870100	25904029	T	1.098	4.596	8,95E-06	12p12.1	No gene
8	rs1025856	106733869	G	-0.5911	-4.578	9,36E-06	8q23.1	ZFPM2

FIGURES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B
Q−Q plot (on chisq[1]) of x1
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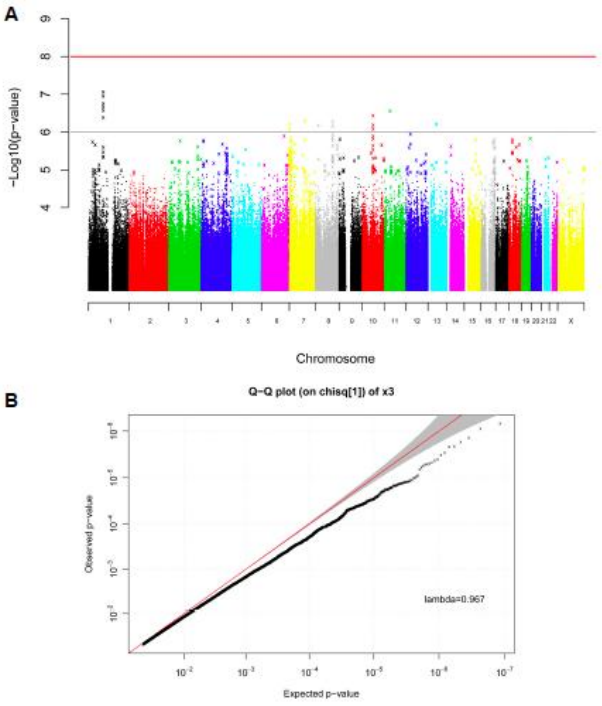
Expected p−value
lambda=1.01


A
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Chromosome
B
Q−Q plot (on chisq[1]) of x3
Observed p−value
Expected p−value
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